

What is claimed is:

- 5 1. A method for immunizing an individual to prevent  
disease caused by a gram-negative bacterial pathogen,  
the method comprising vaccinating the individual with a  
prophylactically effective amount of a vaccine  
formulation comprising an active ingredient selected  
10 from the group consisting of an *htrB* mutant of said  
gram-negative bacterial pathogen, endotoxin isolated  
from the *htrB* mutant of said gram-negative bacterial  
pathogen, endotoxin isolated from the *htrB* mutant of  
said gram-negative bacterial pathogen said endotoxin  
15 conjugated to a carrier protein, and an *htrB* mutant of  
said gram-negative bacterial pathogen which has been  
genetically engineered to express at least one  
heterologous vaccine antigen; wherein said *htrB* mutant  
lacks one or more secondary acyl chains of lipid A  
20 contained in the gram-negative bacterial pathogen  
resulting in substantially reduced toxicity when  
compared to lipid A of the gram-negative bacterial  
pathogen.
- 25 2. The method of claim 1, wherein the individual is a  
human, and the vaccine formulation is introduced by a  
route of administration selected from the group  
consisting of intradermal, intramuscular,  
intraperitoneal, intravenous, subcutaneous, ocular,  
30 intranasal, and oral administration.
3. The method of claim 2, wherein the vaccine  
formulation comprises an active ingredient consisting  
essentially of an *htrB* mutant of said gram-negative  
35 bacterial pathogen.

4. The method of claim 2, wherein the vaccine formulation comprises an active ingredient consisting essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen.
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5. The method of claim 2, wherein the vaccine formulation comprises an active ingredient consisting essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, wherein the isolated endotoxin is conjugated to a carrier protein.
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6. The method of claim 2, wherein the vaccine formulation comprises an active ingredient consisting essentially of an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous antigen from a microbial pathogen.
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7. The method of claim 2, wherein the vaccine formulation further comprises a physiological carrier and an adjuvant.
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8. The method of claim 1, wherein the individual is an animal, and the vaccine formulation is introduced by a route of administration selected from the group consisting of intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, ocular, intranasal, and oral administration.
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9. The method of claim 8, wherein the vaccine formulation comprises an active ingredient consisting essentially of an *htrB* mutant of said gram-negative bacterial pathogen.
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10. The method of claim 8, wherein the vaccine formulation comprises an active ingredient consisting
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essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen.

11. The method of claim 8, wherein the vaccine  
5 formulation comprises an active ingredient consisting essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, wherein the isolated endotoxin is conjugated to a carrier protein.
12. The method of claim 8, wherein the vaccine  
10 formulation comprises an active ingredient consisting essentially of an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous antigen from a  
15 microbial pathogen.
13. The method according to claim 8, wherein the *htrB* mutant of said gram-negative bacterial pathogen is administered orally as an additive to animal feed.  
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14. The method according to claim 12, wherein the *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous antigen from a microbial pathogen is  
25 administered orally as an additive to animal feed.
15. The method of claim 8, wherein the vaccine formulation further comprises a physiological carrier and an adjuvant.  
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16. A vaccine formulation comprising an active ingredient selected from the group consisting of an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of said gram-negative  
35 bacterial pathogen, endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen said

endotoxin conjugated to a carrier protein, and an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous vaccine antigen; wherein said *htrB* mutant  
5 lacks one or more secondary acyl chains of lipid A contained in the gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the gram-negative bacterial pathogen.

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17. The vaccine formulation according to claim 16, wherein the active ingredient consists essentially of an *htrB* mutant of said gram-negative bacterial pathogen.

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18. The vaccine formulation according to claim 16, wherein the active ingredient consists essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen. B

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19. The vaccine formulation of claim 16, wherein the active ingredient consists essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, wherein the isolated endotoxin is conjugated to a carrier protein.

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20. The vaccine formulation according to claim 16, wherein the active ingredient consists essentially of an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at  
30 least one heterologous antigen from a microbial pathogen.

21. The vaccine formulation according to claim 16, further comprising a physiological carrier and an  
35 adjuvant.

22. A method of making in a gram-negative bacterial pathogen a mutant endotoxin of substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen, the method comprising mutating an *htrB* gene within the gram-negative bacterial pathogen, wherein said mutation causes a phenotype of a resultant *htrB* mutant characterized by a mutant endotoxin lacking one or more secondary acyl chains of lipid A contained in the wild type gram-negative bacterial pathogen.

23. A mutant endotoxin of substantially reduced toxicity, made according to the method of claim 22, wherein the mutant endotoxin having substantially reduced toxicity was purified from the *htrB* mutant by a process selected from the group consisting of a phenol/water extraction, and a protease digestion; and wherein the purified mutant endotoxin having substantially reduced toxicity is used to generate endotoxin-specific antibodies.

24. The mutant endotoxin according to claim 23, further comprising conjugation to a carrier protein.

25 25. A mutant endotoxin of substantially reduced  
toxicity, made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, further comprising conjugation to a carrier protein.

27. A method of making an *htrB* mutant of a wild type gram-negative bacterial pathogen wherein the *htrB* mutant has substantially reduced toxicity when compared to the wild type gram-negative bacterial pathogen, the method comprising mutating an *htrB* gene within the gram-negative bacterial pathogen, wherein said mutation

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causes a phenotype of a resultant *htrB* mutant characterized by endotoxin lacking at least one secondary acyl chain on lipid A contained in the wild type gram-negative bacterial pathogen.

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28. A mutant gram-negative bacterial pathogen of substantially reduced toxicity, made according to the method of claim 27, wherein the gram-negative bacterial pathogen having substantially reduced toxicity is used to generate endotoxin-specific antibodies.

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29. A method for producing endotoxin-specific antisera for a use selected from the group consisting of in diagnostic assays, and for passive immunization, the method comprises immunizing an individual with a vaccine formulation comprising an active ingredient selected from the group consisting of an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, and endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen said endotoxin conjugated to a carrier protein; and collecting antibody produced from said immunized individual; wherein said *htrB* mutant lacks one or more secondary acyl chains of lipid A contained in the wild type gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.

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AMENDED SHEET